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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/445, 38/39, 31/335</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/09982</b> <b>(43) International Publication Date:</b> 4 March 1999 (04.03.99)
<b>(21) International Application Number:</b> PCT/US98/17538 <b>(22) International Filing Date:</b> 25 August 1998 (25.08.98)  <b>(30) Priority Data:</b> 60/056,765 25 August 1997 (25.08.97) US  <b>(71)(72) Applicants and Inventors:</b> BREM, Harold [US/US]; Apartment 404, 501 Hicks Street, Brooklyn, NY 11231 (US); EHRLICH, Jason [US/US]; 995 Jonestown Road, P.O. Box 545, Grantville, PA 17028 (US); FOLKMAN, Judah [US/US]; 18 Chatham Circle, Brookline, MA 02146 (US).  <b>(74) Agents:</b> PABST, Patrea, L. et al.; Arnall Golden & Gregory, LLP, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450 (US).		<b>(81) Designated States:</b> AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PREVENTION OF ADHESIONS AND EXCESSIVE SCAR FORMATION USING ANGIOGENESIS INHIBITORS		
<b>(57) Abstract</b>  A method and compositions for inhibiting excessive scar formation and adhesions by administering to a patient in need thereof an effective amount of an angiogenesis inhibitor. In the preferred embodiment, the angiogenesis inhibitor is selective, such as a fumagillol derivative like 0-chloroacetylcarbonyl-Fumagillol (TNP-470; TAP Pharmaceuticals), thalidomide, or a selective drug having more than one activity, such as minocycline or penicillamine which also have antibiotic activity. Less selective compounds can also be used, such as the cytokine IL12. Patients to be treated include those having experienced trauma, surgical intervention, burns, and other types of injuries. The inhibitor is administered in an amount effective to decrease excessive scarring, defined as formation of high density tissue including cells and connective tissue, without preventing normal wound closure. The inhibitors can be administered systemically and/or locally or topically, as needed. For prevention of adhesions, the angiogenesis inhibitor would typically be applied at the time of surgery, preferably in a controlled release formulation and/or using barrier technology.		

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## PREVENTION OF ADHESIONS AND EXCESSIVE SCAR FORMATION USING ANGIOGENESIS INHIBITORS

### Background of the Invention

The present invention relates to methods for the prevention of adhesions, excessive scar formation and other types of abnormal proliferation of tissue using angiogenesis inhibitors.

Scars are the result of wounds that have healed, lesions due to diseases, or surgical operations. Hypertrophic and keloid scars occur when the tissue response is out of proportion to the amount of scar tissue required for normal repair and healing. A keloid scar is a raised, firm, thickened red scar that exceeds the boundary of the injury and may grow for a prolonged period of time. The increase in scar size is due to deposition of an increased amount of collagen into the tissue. African-Americans are genetically prone to developing keloids. Keloid development has been associated with different types of skin injury including surgery, ear piercing, laceration, burns, vaccination or inflammatory process. Common sites are earlobes and the upper trunk and extremities. Surgical removal of keloids alone has been associated with a recurrence rate of 45% to 100%.

The problem of excessive scar formation that manifests itself clinically is a multi-billion dollar problem. For example, intra-abdominal adhesions results in a very significant morbidity and mortality in every surgery practice. Greater than 400,000 hospital admissions in the United States per year are for treatment of pelvic adhesions following surgery. Repeat surgery can greatly aggravate scarring.

There is no effective treatment to prevent adhesions, although numerous different technologies have been tried, including application of barriers which are designed to avoid direct tissue to tissue interactions, and administration of immunosuppressants to decrease inflammatory reactions. Currently there is only one product approved by the FDA for intra-abdominal and one product approved by the FDA for pelvic adhesions. These are principally mechanical barriers to adhesion formation. They are

only minimally effective clinically and there remains a huge demand for an alternative. Keloids have been treated with injection of corticosteroid into the scar, by laser therapy, and by administration of pharmacologic agents that interfere with collagen synthesis. Numerous treatments after excision have been used, such as postexcisional injections of corticosteroids or interferon, silicone sheeting, radiotherapy, and pressure splints or garments. See, for example, Berman, et al., J. Am. Acad. Dermatol. 33, 117-123 (1995); Pulton, Dermatol. Surg. 21, 947-951 (1995); Cosman, et al., Plast. Reconstr. Surg. 53, 540-543 (1974); Rockwell, et al., Plast. Reconstr. Surg. 827-835 (1989); Griffin, et al., Plast. Reconstr. Surg. 46, 145-150 (1970); Bisley, et al., J. Am. Acad. Dermatol. 35, 113-114 (1996); Klumper, et al., J. Acad. Dermatol. 31, 225-231 (1994); Larrabee, et al., Arch. Otolaryngol. Head Neck Surg. 116, 1159-1162 (1990); Darzi, et al., J. Plast. Surg. 45, 374-379 (1992); and Berman, et al., J. Am. Acad. Dermatol. 37, 755-757 (1997).

It is therefore an object of the present invention to provide a treatment for the prevention of excessive scarring and adhesions, without inhibition of wound healing.

#### Summary of the Invention

A method and compositions for inhibiting excessive scar formation and adhesions by administering to a patient in need thereof an effective amount of an angiogenesis inhibitor. In the preferred embodiment, the angiogenesis inhibitor is selective, such as a fumagillol derivative like 0-chloroacetylcarbamoyl-Fumagillol (TNP-470, TAP Pharmaceuticals), thalidomide, or a selective drug having more than one activity, such as minocycline or penicillamine which also have antibiotic activity. Less selective compounds can also be used, such as the cytokine IL12. Patients to be treated include those having experienced trauma, surgical intervention, burns, and other types of injuries. The inhibitor is administered in an amount effective to decrease excessive scarring, defined

as formation of high density tissue including cells and connective tissue, without preventing normal wound closure. The inhibitors can be administered systemically and/or locally or topically, as needed. For prevention of adhesions, the angiogenesis inhibitor would typically be applied at the time of surgery, preferably in a controlled release formulation and/or using barrier technology.

#### Brief Description of the Drawings

Figure 1 is a graph of endogenous basic fibroblast growth factor (bFGF) (pg/cm<sup>2</sup>) over time (days after wounding) of mice treated with TNP-470 (solid circles) and controls that were not treated (open circles).

Figure 2 is a graph of percent wounds open over time (days after wounding) for mice treated with TNP-470 for different dosage regimes: pretreatment days -60 to day 0; treated with TNP-470 days 5 to 18; treated with TNP-470 days 0 to 18; and treated with TNP-470 days 0, 2 and 4.

Figure 3 is a graph of breaking strength (tensile strength) of linear wounds after systemic administration of TNP-470 days -20 to 0; days 0 to 4; days 0 to 12; days 5 to 12; and control, measured on days 4, 7 and 12 after wounding.

#### Detailed Description of the Invention

It has been discovered that excessive scar formation and adhesions can be prevented or inhibited by administration of an effective amount of anti-angiogenesis inhibitors.

Primate skin and soft tissue does not regenerate. Instead function is restored by the deposition of new connective tissue, a scar. The extent of scarring is proportional to and related to the severity of tissue loss (in terms of volume and depth). Commonly, a deep wound involving a significant loss of dermis will heal with the deposition of excess scar, a hypertrophic scar. Another form of excess scarring which is much less common is the keloid scar. The keloid scar exceeds the boundaries of the original injury. Its intrusion into involved surrounding skin differs from

that of a hypertrophic scar which remains within the boundaries of the original injury. Another difference that distinguishes these abnormal scars is the presence of nodules in hypertrophic scars and their absence in keloids. Both abnormal scar conditions are characterized by the excess deposition of connective tissue, composed of a relatively high density of cells, and increased volume of connective tissue matrix and an increased vascular supply resulting from increased number of vessels. Evidence gathered by laser Doppler blood flow monitoring demonstrated that hypertrophic scars developed as a consequence of a defect in the remodeling phase of repair (Ehrlich, H.P., Kelley, S.F. (1992) Plast. Reconstr. Surg. 90:993-998). No evidence was found that supported that the hypertrophic scar was the uninterrupted continuation of the proliferative phase of repair. Keloid scars also exhibit a high density of blood vessels and enhanced circulation within the connective tissue scar tissues (Ehrlich, H.P., et al. (1994) Am. J. Pathol. 145:105-113). Therefore, diminishing blood supply or preventing the development of an increased blood supply with a higher density of blood vessels, will block and retard the development of excess scarring.

Scar formation is the end product of the wound healing process. This process occurs as a series of overlapping phases that occur over a period of time. The initial phase called the lag phase is characterized by the deposition of a transitional matrix mostly composed of fibrin and an inflammatory response. Following the lag phase, there is the proliferative phase of repair which is characterized by the invasion by, and an increased density of, mesenchymal cells, the syntheses and deposition of a new connective tissue matrix, the completion of re-epithelialization and the development of a new blood supply. The remodeling phase of repair terminates the proliferative phase and is characterized by a decrease in cell density, the better organization of the connective tissue matrix, and intact maturing epidermal surface and a decrease in the density of blood vessels. It is clear that without the reduction in blood vessel density, the remodeling

phase of repair is impaired and an immature, excessive fibrotic tissue is deposited within the healed wound site. The nutritional requirement of the mesenchymal cells in excess scar tissue requires an enhanced blood supply. Preventing the development of that blood supply will block the development of excess scarring.

The control of angiogenesis represents the controls of the nutrient supply to the mesenchymal cells responsible for the synthesis and organization of the newly deposited connective tissue matrix of scar or fibrotic tissues. Without an adequate nutrient supply, limitations on the function and synthetic activity of mesenchymal cells occurs. Blocking the progression of angiogenesis will limit the nutrients supplied to mesenchymal cells and limit their metabolic activity.

#### I. Therapeutic Compositions

##### Anti-Angiogenesis Inhibitors

Angiogenesis, the proliferation and migration of endothelial cells that result in the formation of new blood vessels, is an essential event in a wide variety of normal and pathological processes. For example, angiogenesis plays a critical role in embryogenesis, wound healing, psoriasis, diabetic retinopathy, and tumor formation, as reported by Folkman, J. Angiogenesis and its inhibitors. In: V. T. DeVita, S. Hellman and S. A. Rosenberg (eds.), Important Advances in Oncology, pp. 42-62. (J. B. Lippincott Co., Philadelphia, 1985); Brem, H., et al., Brain tumor angiogenesis. In: P. L. Kornblith and M. D. Walker (eds.), Advances in Neuro-Oncology, pp. 89-101. (Future Publishing Co., Mount Kisco, NY 1988); Folkman, J. Tumor angiogenesis: therapeutic implications. N. Engl. J. Med., 285: 1182-1186 (1971); and Folkman, J. Successful treatment of an angiogenic disease. N. Engl. J. Med., 320: 1211-1212 (1989).

Identification of several agents that inhibit tumor angiogenesis has provided a conceptual framework for the understanding of angiogenesis in



general. The inhibition of angiogenesis by certain steroids and heparin derivatives, reported by Folkman, J., et al., Science 221: 719 (1983); and Murray, J. B., et al., Purification and partial amino acid sequence of a bovine cartilage-derived collagenase inhibitor. J. Biol. Chem., 261: 4154-4159 (1986); led to studies elucidating the crucial role of remodeling of the extracellular matrix in angiogenesis. These agents apparently prevent angiogenesis by specifically disrupting the deposition and cross-linking of collagen, as reported by Ingber, D., and Folkman, J. Inhibition of angiogenesis through modulation of collagen metabolism. Lab. Invest., 59: 44-51 (1989).

The original description of angiogenesis inhibition in the presence of cartilage, reported by Brem, H., et al., J. Exp. Med. 141: 427-439 (1975); Brem, H., et al., Extracellular Matrices Influences on Gene Expression pp. 767-772 (Academic Press, NY 1975); and Langer, R., et al., Science 70-72 (1976); led to the isolation and purification from bovine cartilage of a protein fraction that not only inhibited angiogenesis but inhibited protease activity, described by Murray, J.B., et al., J. Biol. Chem. 261: 4154-4159 (1986). Subsequently, an extract derived from the vitreous of rabbits was shown to inhibit tumor angiogenesis by Brem, S., et al., Am. J. Ophthal. 84: 323-328 (1977). The demonstration that heparin alone enhanced the angiogenesis response buttressed the hypothesis that heparin produced by mast cells that had migrated to the tumor site facilitated the development of new capillaries, as reported by Kessler, D.A., et al., Int. J. Cancer 18:703-709 (1976).

Other studies on inhibition of angiogenesis have highlighted the importance of enzyme mediated remodeling of the extracellular matrix in capillary growth and proliferation (Folkman, J., et al., Science 221: 719-725 (1983); Ingber, D., et al. Lab. Invest. 59: 44-51 (1989); Folkman, J., et al., Science 243: 1490-1493 (1989); Krum, R., et al., Science 230: 1375-1378 (1985); Ingber, D., et al., Endocrinol. 119: 1768-1775 (1986); and Ingber, D., et al., J. Cell. Biol. 109: 317-330 (1989)). A number of



investigators have reported that extracts of cartilage, one of the few avascular tissues in the body, can inhibit angiogenesis: Eiseinstein, et al., Am. J. Pathol. 81, 1-9 (1987); Pauli, et al., J. Natl. Cancer Inst. 67, 55-74 (1981); Brem and Folkman, J. Exp. Med. 141, 427-439 (1975); Langer, et al., Science 193, 70-72 (1976); Langer, et al., Proc. Natl. Acad. Sci. USA 77, 431-435 (1980); and Lee and Langer, Science 221, 1185-1187 (1983).

Several different classes of compounds have been determined to be useful as inhibitors of angiogenesis. These include collagenase inhibitors such as metalloproteinases and tetracyclines such as minocycline, naturally occurring peptides such as endostatin and angiostatin, described for example in U.S. patent No. 5,733,876 to O'Reilly, et al., U.S. patent No. 5,290,807, and U.S. Patent No. 5,639,725, fungal and bacterial derivatives, such as fumagillin derivatives like TNP-470, the sulfated polysaccharides described in U.S. patent No. 4,900,815 to Tanaka, et al. and the protein-polysaccharides of U.S. patent No. 4,975,422 to Kanoh, et al. and synthetic compounds such as the 2,5-diaryltetrahydrofurans of U.S. patent No. 5,629,340 to Kuwano, et al., aminophenylphosphonic acid compounds of U.S. patent No. 5,670,493 to Cordi, et al., the 3-substituted oxindole derivatives of U.S. patent No. 5,576,330 to Buzzetti, et al., and thalidomides of U.S. patent No. 5,712,291 to D'Amato.

The antibiotics that are useful as angiogenesis inhibitors are those having collagenase inhibitory activity. These include the tetracyclines and chemically modified tetracyclines (CMTs), and three ringed tetracycline homologs, that have the ability to inhibit collagenase but diminished antibacterial activity. Examples of commercially available tetracyclines include chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, rolitetracycline, and tetracycline. The active salts, which are formed through protonation of the dimethylamino group on carbon atom 4, exist as crystalline compounds. These are stabilized in aqueous solution by addition of acid.

Minocycline, a semisynthetic tetracycline antimicrobial, described by Martell, M. J., and Boothe, J. H. in J. Med. Chem., 10: 44-46 (1967), and Zbinovsky, Y., and Chrikian, G. P. Minocycline. In: K. Florey (ed.), Analytical Profiles of Drug Substances, pp. 323-339 (Academic Press, NY 1977), the teachings of which are incorporated herein, has anticollagenase properties, as reported by Golub, L. M., et al., J. Periodontal Res., 18: 516-526 (1983); Golub, L. M., et al., J. Periodontal Res., 19: 651-655 (1984); Golub, L. M., et al., J. Periodontal Res., 20: 12-23 (1985); and Golub, L. M., et al., J. Dent. Res., 66: 1310-1314 (1987). Minocycline, first described in 1967, is derived from the naturally produced parent compounds chlortetracycline and oxytetracycline. The chemically modified tetracyclines are described by U.S. Patent No. 4,704,383 to McNamara, et al.; 4,925,833 to McNamara, et al.; and 4,935,411 to McNamara, et al.; the teachings of which are incorporated herein.

Other exemplary anti-angiogenic compounds include penicillamine and some cytokines such as IL12.

#### B. Carriers

Pharmaceutical compositions containing the angiogenesis inhibitor are prepared based on the specific application. Application can be either topical, localized, or systemic. Any of these compositions may also include preservatives, antioxidants, antibiotics, immunosuppressants, and other biologically or pharmaceutically effective agents which do not exert a detrimental effect on the normal tissue to be treated.

Compositions for local or systemic administration will generally include an inert diluent. Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating

agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

#### Systemic Carriers

Inhibitors can be systemically administered either parenterally or enterally. The composition can be administered by means of an infusion pump, for example, of the type used for delivering insulin or chemotherapy to specific organs or tumors, by injection, or by depo using a controlled or sustained release formulation. In a preferred systemic embodiment, drugs are administered orally, in an enteric carrier if necessary to protect the drug during passage through the stomach.

The angiogenic inhibitors can be administered systemically by injection in a carrier such as saline or phosphate buffered saline (PBS) or orally, in the case of an inhibitor such as thalidomide, in tablet or capsule form. The tablets or capsules can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; or a glidant such as colloidal silicon dioxide. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

#### Local or Topical Carriers

The angiogenic inhibitors can also be applied locally or topically, in a carrier such as saline or PBS, in an ointment or gel, in a transdermal patch or bandage, or controlled or sustained release formulation. Local administration can be by injection at the site of the injury, or by spraying

topically onto the injury. The inhibitor can be absorbed into a bandage for direct application to the wound, or released from sutures or staples at the site. Incorporation of compounds into controlled or sustained release formulations is well known.

For topical application, the angiogenesis inhibitor is combined with a carrier so that an effective dosage is delivered, based on the desired activity, at the site of application. The topical composition can be applied to the skin for treatment of diseases such as psoriasis. The carrier may be in the form of an ointment, cream, gel, paste, foam, aerosol, suppository, pad or gelled stick. A topical composition for use of an ointment or gel consists of an effective amount of angiogenesis inhibitor in a ophthalmically acceptable excipient such as buffered saline, mineral oil, vegetable oils such as corn or arachis oil, petroleum jelly, Miglyol 182, alcohol solutions, or liposomes or liposome-like products.

In a preferred form for controlled release, the composition is administered in combination with a biocompatible polymeric implant which releases the angiogenesis inhibitor over a controlled period of time at a selected site. Examples of preferred biodegradable polymeric materials include polyanhydrides, polyorthoesters, polyglycolic acid, polylactic acid, polyethylene vinyl acetate, and copolymers and blends thereof. Examples of preferred non-biodegradable polymeric materials include ethylene vinyl acetate copolymers. These can be prepared using standard techniques as microspheres, microcapsules, tablets, disks, sheets, and fibers.

#### C. Other Therapeutic Agents which can be Administered in Combination

The angiogenic inhibitors can be administered alone or in combination with other treatments. For example, the inhibitors can be administered with antibiotics, cytokines, and antiinflammatories such as cortisone, and/or other types of angiogenic inhibitors. Other combinations will be apparent to those skilled in the art. In a preferred embodiment for prevention of adhesions, the angiogenesis inhibitor is administered with a

barrier, such as methylcellulose or other polymeric material, either topically at the time of surgery or incorporated into the barrier, which is inserted at the time of surgery. In some cases the combination therapy will be achieved through the selection of the angiogenesis inhibitor, for example, minocycline, which both inhibits collagenase and is an antibiotic. One of the causes of the failure of intestinal anastomosis is the breakdown of connective tissue by enhanced collagenase activity. The inhibition of this collagenase activity may be beneficial or improve the outcome of these surgical procedures.

## II. Methods of Treatment

### A. Disorders to be Treated

The angiogenic inhibitors can be used to prevent or inhibit excessive scar formation, especially hypertrophic scars and keloid scars, and adhesions, especially intra-peritoneal or pelvic adhesions such as those resulting after open or laproscopic surgery, and burn contractions. Other conditions which should be beneficially treated using the angiogenesis inhibitors include prevention of scarring following transplantation, cirrhosis of the liver, pulmonary fibrosis following acute respiratory distress syndrom or other pulmonary fibrosis of the newborn, implantation of temporary prosthetics, and adhesions after surgery between the brain and the dura.

### B. Effective Dosages and Treatment Regimes

The angiogenesis inhibitor will typically be administered either at the time of surgery or shortly thereafter, usually within one week, except in those cases where the injury is to the skin or other superficial surface and can be readily accessed following the injury or surgery. A particular example of the latter is in the treatment of wounds. It is desirable to treat exposed wounds of the skin after there has been re-epithelialization of the skin's surface. Wounds in normal healthy patients may close in as short as five to seven days; in deep burn patients wounds may take three to six

weeks to close. Since the remodeling phase of repair occurs after the wound is closed, and there is a new epidermal surface, preventing the new growth of blood vessels at this time is not detrimental to the healing process and will reduce normal skin-like barrier function no more than that of a normal scar. In the case of adhesions, the deposition of connective tissue between normal anatomical structures is unnecessary. Hence preventing the development of an enhanced blood supply to that tissue would not be detrimental to the host. On the other hand, preventing the closure of an open wound could be detrimental to the host. Therefore the time of commencement of therapy would be different in preventing adhesions and preventing excess dermal scarring. In the case of adhesions, therapy will start early, that is, soon after procedures which lead to local trauma and the deposition of a transitional matrix. On the other hand, with skin injury, the healing process would continue and therapy would be withheld until the wound was closed, as demonstrated by an intact epidermal surface.

The angiogenesis inhibitor is administered in a dosage and in a regimen that does not prevent wound healing, but does decrease the amount of blood vessel growth at the wound site to prevent or decrease formation of high density cellular and connective tissue within the scar or outside of the wound area (keloids). In order to have increased levels of cells and deposited connective tissue one must have an increased nutritional supply via vascularization. Dosages will typically be in the same range as used for inhibition of tumor growth, but administered to a different class of patients and for different time periods, since wound healing typically occurs over a much shorter time. Moreover, when administered topically or in a sustained release formulation, the dosage may be lower in order not to prevent wound healing.

The present invention will be further understood by reference to the following non-limiting examples.

**Example 1: Inhibition of Scar Formation**

TNP-470 interferes with blood vessel and collagen formation resulting in decreased adhesions. As demonstrated by the following data, TNP-470 results in the abolishment of intra-abdominal adhesions.

**Methods**

Full thickness mouse wounds were made in adult mice, the majority of whom were six to eight weeks old and some of whom were fourteen to sixteen weeks old. Mice were pretreated for sixty days with TNP-470, then wounds were made, and healing monitored. Mice were treated with 30 mg/kg TNP-470 administered subcutaneously every other day.

Histological micrographs of open mouse wounds harvested at 7, 12, and 17 days post excision were made. The biopsies were fixed, embedded, sectioned and stained with hematoxylin and eosin.

**Results**

Examination of 7-day untreated healing wound granulation tissue showed a high density of blood vessels with some large patent vessels.

Examination of 7-day TNP-470 treated healing wound granulation tissue showed a relatively lower density of blood vessels, with some having smaller patent vessels.

Examination of a 12-day untreated closed wound showed granulation tissue under a re-epithelialized surface with numerous patent blood vessels.

Examination of a 12-day TNP-470 treated healing wound that is not completely closed, showed that granulation tissue under a re-epithelialized surface shows fewer vessels with smaller diameters.

Examination of a 17-day untreated healed wound showed mature granulation tissue with patent vessels, but a reduced density of mesenchymal cells.

Examination of a 17-day TNP-470 treated healed wound showed mature granulation tissue with a reduction in the number of vessels, but a continued higher density of mesenchymal cells.



The histologic findings demonstrate that TNP-470 severely retarded scar formation. At 7 days the treatment group had a lower density of blood vessels and reduced granulation tissue compared to untreated controls. In contrast, mesenchymal cell infiltration was similar in treated compared to untreated animals. At 12 days the open wounds in the controls were completely re-epithelialized and revealed a greater density of patent vessels, compared to partially closed TNP 470-treated wound. In addition, the density of mesenchymal cells in the TNP-470 treated granulation tissue appeared greater than in the controls and the epidermal surface was thicker in the treated group. At 17 days, when both treated and untreated mouse wounds were closed, the density of blood vessels remained elevated in the untreated mice, compared to the treated mice which had a lower density of blood vessels. In contrast, at day 17 after wounding, the density of mesenchymal cells and the thickness of the epidermis was greater in TNP-470 treated mice. Thicker epidermis and greater density of mesenchymal cells represent retarded scar maturation. Thus inhibition of angiogenesis by TNP-470 resulted in retarded wound closure by wound contraction and re-epithelialization and also increased time required for maturation of granulation tissue.

#### Example 2: Inhibition of Scarring during Wound Healing.

Further experiments were conducted to examine the role of the angiogenic inhibitors in wound healing and prevention of excessive scarring.

##### Methods

Mice were treated essentially the same as described in Example 1.

##### Results

As demonstrated by Figure 1, endogenous synthesis of basic fibroblast growth factor in the wound is decreased after treatment with the angiogenesis inhibitor, TNP-470. Treatment of mice with the angiogenesis inhibitor TNP-470 resulted in an average of 37% reduction of bFGF

concentration on day 10 after wound ( $p \leq 0.05$ ). This data suggests that one possible mechanism of TNP-470's ability to inhibit angiogenesis is as a consequence of the decreased amount of basic fibroblast growth factor in the wound.

Histological analysis of the wounds in the control and treated mice compared contraction of full thickness wounds in mice treated with systemic TNP-470 (AGM 1470), every other day after the wound is made, with untreated mice. Treatment with TNP-470 every other day after the wound is made (at a dose identical to that which results in maximal tumor inhibition) causes a 5-day delay in the complete contraction of the wound.

Figure 2 (n=440 mice) compares wound closure following different treatment regimes. TNP-470 inhibited or slowed wound healing by 5 days to full contraction. This was statistically significant. The wound healing remained inhibited if TNP-470 was given on post wound days 0, 2, 4, or post wound days 0, 2, 4, 6, 8, and 10.

Figure 3 (n=280 mice) graphs breaking strength of linear wounds after systemic administration of TNP-470. Breaking strength was inhibited 36% on post wound day 7 and 43% on post wound day 12. This was statistically significant. The wounds were inhibited if TNP-470 was given on post wound days 0, 2, 4 or post wound days 0, 2, 4, 6, 8, and 10.

We claim:

1. A method to inhibit excessive scar formation and adhesions comprising administering to a patient in need thereof an effective amount of an angiogenesis inhibitor.
2. The method of claim 1 wherein the angiogenesis inhibitor is administered systemically.
3. The method of claim 1 wherein the angiogenesis inhibitor is administered topically or locally at the site of a wound.
4. The method of claim 1 wherein the angiogenesis inhibitor is administered to prevent or inhibit formation of hypertrophic scarring.
5. The method of claim 1 wherein the angiogenesis inhibitor is administered to prevent or inhibit formation of keloid scarring.
6. The method of claim 1 wherein the angiogenesis inhibitor inhibits collagenase.
7. The method of claim 1 wherein the angiogenesis inhibitor is selected from the group consisting of endostatin and angiostatin.
8. The method of claim 1 wherein the angiogenesis inhibitor is thalidomide.
9. The method of claim 1 wherein the angiogenesis inhibitor is a fumigillin.
10. A composition for application to a wound comprising an angiogenesis inhibitor in a dosage formulation effective to prevent or inhibit formation of hypertrophic scars or keloid scars.
11. The composition of claim 10 wherein the angiogenesis inhibitor is in a pharmaceutically acceptable carrier for topical application of the inhibitor, selected from the group consisting of an ointment, gel, spray, and paste.
12. The composition of claim 10 wherein the angiogenesis inhibitor is thalidomide in a carrier for topical administration.

13. The composition of claim 10 wherein the angiogenesis inhibitor is in a controlled release formulation which releases an effective amount of angiogenesis inhibitor to prevent or inhibit hypertrophic scar or keloid scar formation over the time period required for healing of a wound.
14. The composition of claim 10 wherein the angiogenesis inhibitor is in a formulation delivering sustained release of an effective amount of angiogenesis inhibitor to prevent or inhibit hypertrophic scar or keloid scar formation over the time period required for healing of a wound.
15. The composition of claim 10 in a dosage not inhibiting normal wound healing.

1/2

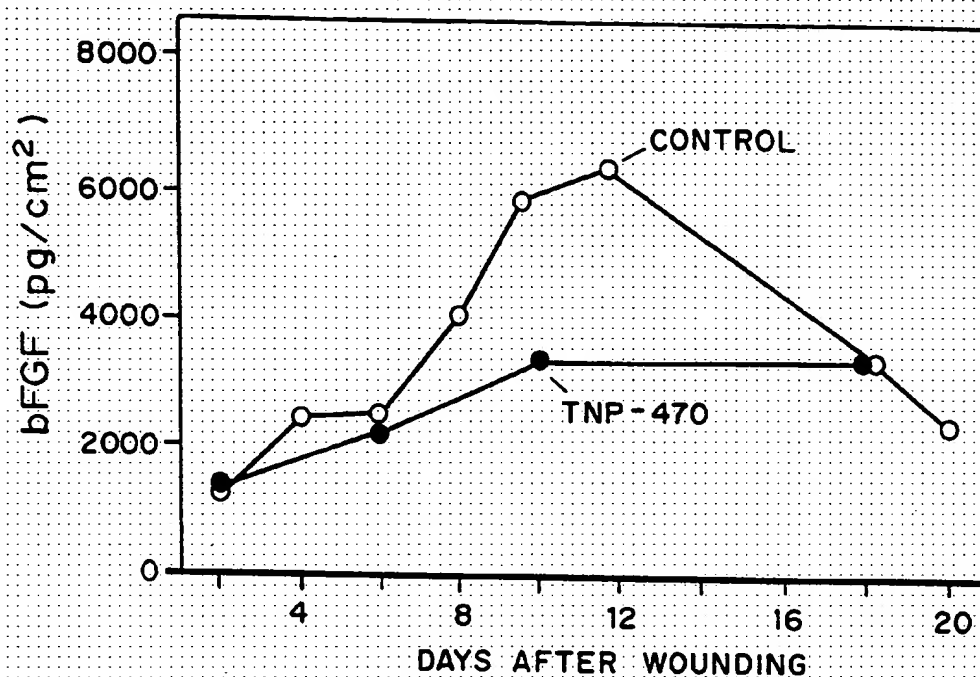


FIG. 1

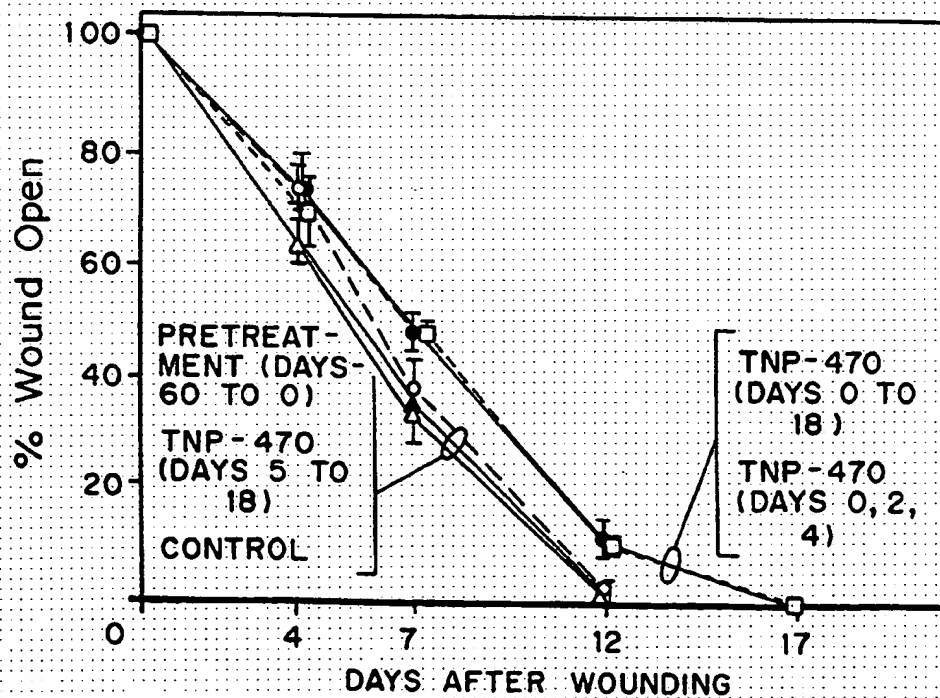


FIG. 2

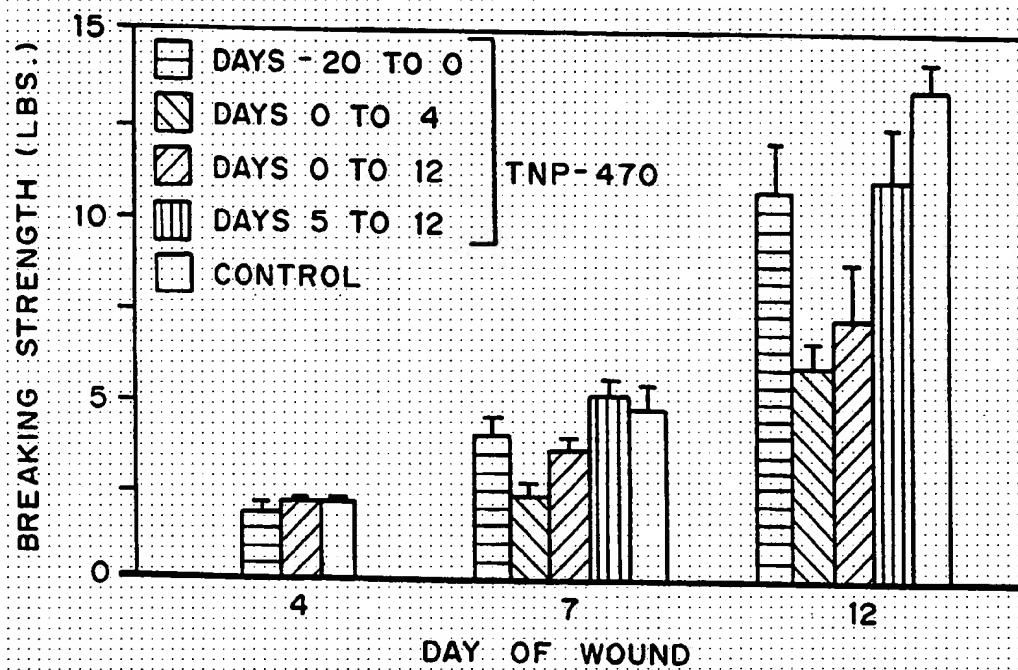


FIG. 3

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US 98/17538

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC 6 A61K31/445 A61K38/39 A61K31/335		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 04142 A (HYBRIDON INC) 9 February 1995 see page 4	1,4,5
P,X	US 5 792 845 A (O'REILLY ET AL.) 11 August 1998 see column 4 - column 6	1,4-7
X	EP 0 325 199 A (TAKEDA) 26 July 1989 see the whole document	1,3,4, 9-12,15
P,X	WO 97 15666 A (CHILDREN'S MEDICAL CENTER) 1 May 1997 see page 6, claim 20 see page 22	1,4,7
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
<b>* Special categories of cited documents:</b> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
3 December 1998		10/12/1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Klaver, T



# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US: 98/17538

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.L. ARBISER: "Angiogenesis and the skin: a primer." J. AM. ACAD. DERMATOL., vol. 34, no. 3, 1996, pages 486-497, XP000673602 see the whole document -----	1,7-9
X	US 5 605 684 A (PIACQUADIO) 25 February 1997 see the whole document -----	10-15

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 17538

## Box I : Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-9  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-9  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II : Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/17538

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504142 A	09-02-1995	AU 7516894 A	28-02-1995
		CA 2167680 A	09-02-1995
		CN 1131437 A	18-09-1996
		EP 0711343 A	15-05-1996
		FI 960374 A	25-03-1996
		JP 9503651 T	15-04-1997
		NO 960303 A	13-03-1996
		US 5731294 A	24-03-1998
		US 5639872 A	17-06-1997
		US 5814620 A	29-09-1998
		US 5801156 A	01-09-1998
		US 5710136 A	20-01-1998
		US 5661135 A	26-08-1997
		US 5639736 A	17-06-1997
		US 5641756 A	24-06-1997
US 5792845 A	11-08-1998	US 5639725 A	17-06-1997
		AU 692865 B	18-06-1998
		AU 2461795 A	16-11-1995
		BR 9507479 A	16-09-1997
		CA 2188813 A	02-11-1995
		CN 1149319 A	07-05-1997
		CZ 9603122 A	14-05-1997
		EP 0758390 A	19-02-1997
		HU 76095 A	30-06-1997
		JP 9512173 T	09-12-1997
		NZ 285501 A	26-02-1998
		WO 9529242 A	02-11-1995
		US 5733876 A	31-03-1998
		US 5776704 A	07-07-1998
		ZA 9503419 A	11-01-1996
EP 325199 A	26-07-1989	AU 3032789 A	11-08-1989
		CA 1333363 A	06-12-1994
		CA 1330943 A	26-07-1994
		CN 1036135 A, B	11-10-1989
		DE 68910113 D	25-11-1993
		DE 68910113 T	17-03-1994
		DE 68910138 D	02-12-1993
		DE 68910138 T	28-04-1994
		DK 171390 A	21-08-1990
		EP 0398925 A	28-11-1990
		ES 2059571 T	16-11-1994
		GR 1000597 B	26-08-1992
		IE 64346 B	26-07-1995
		JP 1279828 A	10-11-1989
		JP 2806454 B	30-09-1998
		JP 3502323 T	30-05-1991
		US 5637575 A	10-06-1997
		WO 8906536 A	27-07-1989
		US 5135919 A	04-08-1992
		US 5760015 A	02-06-1998
		US 5019562 A	28-05-1991
WO 9715666 A	01-05-1997	AU 7466696 A	15-05-1997
		CZ 9801177 A	12-08-1998
		EP 0857210 A	12-08-1998
		NO 981803 A	17-06-1998

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 98/17538

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5605684 A	25-02-1997	US 5443824 A	22-08-1995
		AU 1997795 A	03-10-1995
		WO 9524891 A	21-09-1995